

020889-000001_Seq_List_ST25.txt
SEQUENCE LISTING

<110> de Lorenzo Prieto, Victor
Fernandez Herrero, Luis A

<120> System for the Production of Dimeric Proteins Based on the
Transport System of Hemolysin of Escherichia Coli

<130> 020884-000001

<140> 10/566,827
<141> 2006-01-31

<150> P200301830 (ES)
<151> 2003-07-31

<150> PCT/ES2004/070053
<151> 2004-07-19

<160> 22

<170> PatentIn version 3.4

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<223> EHlyA polypeptide containing 23 kDa ('hlyA) secretion signal of
E. coli Hly transporter tagged with the E epitope.

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Ser Leu Ala Lys
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<220>
<223> ZEHlyA polypeptide containing the 23 kDa ('hlyA) secretion signal
of E. coli Hly transporter tagged with the E epitope.

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Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Leu Glu
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Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu
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His His His Ser Thr Pro Gly Gly Ala Pro
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<223> Ampicillin resistant plasmid pZEHlyA (sense strand); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa C domain of HlyA

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Leu Leu Asp Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly
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130 135 140

Asp Glu Gly Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe
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Arg Asp Val Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys
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195 200 205

Glu Gln Ile Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu
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Lys Lys Ala Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val
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Tyr Gly His Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro
245 250 255

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<220>
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<210> 7
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 <223> Ampicillin resistant plasmid pZEHLA2SD (missense strand); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA.

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ccttggccgg	ccccttttaa
gttcaacatg	ccgtcactcc
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aataagaagt	cctataccgg	tagtataata		540
gtcaaatcga	ctatatctaa	aggccctgca		600
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<220>
 <223> Ampicillin resistant plasmid pZEHLA2SD (protein); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA'.

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 35 40 45

Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His His His
 Page 7

50

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 Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly Asn Asp Ile
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 Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp Asp Glu Gly
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 Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp Val
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 Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Glu Gly
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 Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn Trp
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 195 200 205
 Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys Ala
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 Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys Glu
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<210> 9
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<223> Ampicillin resistant plasmid pVamyHLYA (sense strand) containing amplified DNA product encoding VHH amylase (Vamy); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in

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<210> 11
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<212> PRT
<213> Artificial

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<223> Amp-r plasmid pVamyHLYA (protein) containing amplified DNA product encoding VHH amylase (Vamy); 23-kDa C-terminal domain of HLYA with E-tag epitope incorporated at the 23-kDa domain of HLYA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA

<400> 11

Met Ala Gln Val Gln Leu Val Glu Ser Trp Gly Gly Ser Val Gln Ala
1 5 10 15

Gly Gly Ser Leu Arg Leu Ser Cys Thr Ala Pro Gly Phe Thr Ser Asn
20 25 30

Ser Cys Arg Met Asp Trp Tyr Arg Gln Ala Ala Gly Lys Gln Arg Glu
35 40 45

Trp Val Ser Ser Ile Ser Thr Asp Gly Arg Thr Ser Tyr Ala Asp Ser
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Lys Asp Lys Ala Lys Asp Thr Val
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr
85 90 95

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Cys Ala Val Arg Thr Asn Gly Tyr Arg Pro Gln Ser His Glu Phe Arg
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Tyr Trp Gly Pro Gly Thr Gln Val Thr Val Ser Ser Thr Ala Ser Gly
115 120 125

Ala Ala Ser Thr Pro Gly Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu
130 135 140

Glu Pro Ala Gly Glu Asn Ser Leu Ala Lys Asn Val Leu Ser Gly Gly
145 150 155 160

Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp Leu Leu Asp
165 170 175

Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly Asn Asp Ile
180 185 190

Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp Asp Glu Gly
195 200 205

Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp Val
210 215 220

Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Glu Gly
225 230 235 240

Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn Trp
245 250 255

Phe Glu Lys Glu Ser Asp Asp Leu Ser Asn His Gln Ile Glu Gln Ile
260 265 270

Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys Ala
275 280 285

Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val Tyr Gly His
290 295 300

Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro Leu Ile Asn
305 310 315 320

Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys Glu
325 330 335

Glu Arg Ser Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn Ala Ser Asp
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020889-000001_seq_List_ST25.txt

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<210> 12
<211> 2963
<212> DNA
<213> Artificial

<220>
<223> Amp-r plasmid pVamyZHLA (sense strand) containing amplified DNA product encoding VHH amylase; 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA

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<210> 13
 <211> 2963
 <212> DNA
 <213> Artificial

<220>
 <223> Amp-r plasmid pVamyZHLA (missense strand) containing amplified DNA product encoding VHH amylase; 23-kDa C-terminal domain of
 Page 15

020889-000001_Seq_List_ST25.txt
HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA
and polylinker for cloning of scFv's in frame with E-tagged 'hlyA

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<210> 14
 <211> 423
 <212> PRT
 <213> Artificial

<220>
 <223> Amp-r plasmid pVamyZHLA (protein) containing amplified DNA product encoding VHH amylase (Vamy); 23-kDa C-terminal domain of HLYA with E-tag epitope incorporated at the 23-kDa domain of HLYA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA

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 20 25 30

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Ser Cys Arg Met Asp Trp Tyr Arg Gln Ala Ala Gly Lys Gln Arg Glu
35 40 45

Trp Val Ser Ser Ile Ser Thr Asp Gly Arg Thr Ser Tyr Ala Asp Ser
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Lys Asp Lys Ala Lys Asp Thr Val
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr
85 90 95

Cys Ala Val Arg Thr Asn Gly Tyr Arg Pro Gln Ser His Glu Phe Arg
100 105 110

Tyr Trp Gly Pro Gly Thr Gln Val Thr Val Ser Ser Thr Ala Ser Gly
115 120 125

Ala Ala Ser Thr Ser Gly Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser
130 135 140

Ser Arg Met Lys Gln Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys
145 150 155 160

Asn Tyr His Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly
165 170 175

Glu Arg Gly Gly His His His His His Ala Ser Thr Pro Gly Gly
180 185 190

Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Ala Gly Glu Asn Ser
195 200 205

Leu Ala Lys Asn Val Leu Ser Gly Gly Lys Gly Asn Asp Lys Leu Tyr
210 215 220

Gly Ser Glu Gly Ala Asp Leu Leu Asp Gly Gly Glu Gly Asn Asp Leu
225 230 235 240

Leu Lys Gly Gly Tyr Gly Asn Asp Ile Tyr Arg Tyr Leu Ser Gly Tyr
245 250 255

Gly His His Ile Ile Asp Asp Glu Gly Gly Lys Asp Asp Lys Leu Ser
260 265 270

Leu Ala Asp Ile Asp Phe Arg Asp Val Ala Phe Lys Arg Glu Gly Asn
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020889-000001_Seq_List_ST25.txt

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Lys Asn Gly Ile Thr Phe Lys Asn Trp Phe Glu Lys Glu Ser Asp Asp
305 310 315 320

Leu Ser Asn His Gln Ile Glu Gln Ile Phe Asp Lys Asp Gly Arg Val
325 330 335

Ile Thr Pro Asp Ser Leu Lys Lys Ala Phe Glu Tyr Gln Gln Ser Asn
340 345 350

Asn Lys Val Ser Tyr Val Tyr Gly His Asp Ala Ser Thr Tyr Gly Ser
355 360 365

Gln Asp Asn Leu Asn Pro Leu Ile Asn Glu Ile Ser Lys Ile Ile Ser
370 375 380

Ala Ala Gly Asn Phe Asp Val Lys Glu Glu Arg Ser Ala Ala Ser Leu
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 <211> 218
 <212> PRT
 <213> Escherichia coli

<400> 16

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 20 25 30

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 35 40 45

Ser Gly Tyr Gly His His Ile Ile Asp Asp Glu Gly Gly Lys Asp Asp
 50 55 60

Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp Val Ala Phe Lys Arg
 65 70 75

Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Gly Asn Val Leu Ser
 85 90 95

Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn Trp Phe Glu Lys Glu
 100 105 110

Ser Asp Asp Leu Ser Asn His Gln Ile Glu Gln Ile Phe Asp Lys Asp
 115 120 125

Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys Ala Phe Glu Tyr Gln
 130 135 140

Gln Ser Asn Asn Lys Val Ser Tyr Val Tyr Gly His Asp Ala Ser Thr
 145 150 155 160

Tyr Gly Ser Gln Asp Asn Leu Asn Pro Leu Ile Asn Glu Ile Ser Lys
 165 170 175

Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys Glu Glu Arg Ser Ala
 180 185 190

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 195 200 205

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210

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<213> Artificial

<220>
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30

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<220>
<223> Antibody Hinge Region (Protein)

<400> 18

Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser
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<213> Artificial

<220>
<223> BACKHINGE Primer

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33

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<212> DNA
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<212> DNA
<213> Artificial

<220>
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Page 21

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<211> 48
<212> DNA
<213> Artificial

<220>
<223> VHHASfil primer

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48